

**BRIEF REPORT****Effect of Vitamin B<sub>12</sub> and Folic Acid Deficiencies on Neutrophil Function**

By Sandra S. Kaplan and R. E. Basford

Morphological and quantitative neutrophil abnormalities are common in the megaloblastic anemias of vitamin B<sub>12</sub> and folic acid deficiency. Little is known, however, about the role of these vitamins in normal leukocyte function. Seven patients with megaloblastic bone marrows, four with vitamin B<sub>12</sub> deficiency and three with folic acid deficiency, were studied to determine the effect, if any, of these deficiencies on leukocyte function. Phagocytosis of staphylococci, hexose monophosphate shunt activation with phagocytosis, and microbicidal capacity against *Staphylococcus aureus* were determined prior to the institution of specific therapy. In two instances, these studies were repeated

following treatment. There was no impairment of phagocytosis per se, and resting metabolism was not significantly decreased. With phagocytosis, however, metabolic activation was decreased to 35%–36% of control values in the leukocytes of patients with vitamin B<sub>12</sub> deficiency but not in the leukocytes of patients with folic acid deficiency. Bacterial killing was slightly decreased in vitamin B<sub>12</sub> but not in folic acid deficiency. These abnormalities of function were reversed after specific therapy. These findings suggested a specific role for vitamin B<sub>12</sub> in the production of intermediates necessary for normal cell function.

**T**HE MEGALOBLASTIC ANEMIAS of vitamin B<sub>12</sub> and folic acid deficiencies are associated with qualitative and quantitative abnormalities of developing and mature polymorphonuclear (PMN) leukocytes. Such deficiencies usually are associated with macrocytic PMN precursors and reduced numbers of circulating PMN leukocytes with hypersegmented nuclei.<sup>1</sup>

Relatively little research has been done on the role of vitamin B<sub>12</sub> and folic acid in leukocyte metabolism or function, but the macrocytosis and nuclear hypersegmentation of PMN leukocytes generally has been believed to be related to impaired DNA synthesis.<sup>2</sup> Vitamin B<sub>12</sub> coenzyme also serves as a cofactor for the production of succinyl CoA from methylmalonyl CoA and, in deficiency states, leukocyte propionate oxidation is reduced.<sup>3,4</sup> These facts do not themselves suggest that leukocyte function may be impaired. However, the disordered morphology of developing and mature leukocytes, as well as the known impairment of leukocyte function in protein-calorie malnutrition<sup>5,6</sup> and the qualitative platelet defect in vitamin B<sub>12</sub> deficiency<sup>7,8</sup> provoked this investigation.

The leukocyte function of seven patients, three with folic acid deficiency and four with vitamin B<sub>12</sub> deficiency, was evaluated prior to the institution of specific therapy. In several instances, studies were repeated after reversal of the anemia. This evaluation included efficiency of phagocytosis, the phagocytosis-associated activation of the hexose monophosphate shunt (HMPS) and micro-

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Table 1. Hexose Monophosphate Shunt Activity\*

Conditions		Resting	Phagocytosis Associated	
			Latex	Staph
Vitamin B <sub>12</sub> deficiency	C.J.	4.5	136	36
	D.C.	4.4	39	10
	R.R.	0.6	125	45
	E.H.	9.1	108	57
Mean		4.6 ± 2	102 ± 26	37 ± 12
p		<0.2	<0.001	<0.005
Folic acid deficiency	C.W.	6.6	295	161
	E.M.	6.5	302	170
	N.J.	2.8	185	93
Mean		5.3 ± 1.25	261 ± 38.15	141 ± 24.34
p		<0.3	>0.5	<0.5
Normal values		9.9 ± 1.3	249 ± 12.9	104 ± 8.4
n		23	23	18

\*Nanomoles <sup>14</sup>CO<sub>2</sub> produced from 1-<sup>14</sup>C glucose per 10<sup>7</sup> leukocytes per 30 min; mean ± SE.

bicidal capacity against *Staphylococcus aureus*. Our results indicate that, although leukocytes appeared to be quantitatively and morphologically similar in vitamin B<sub>12</sub> and folic acid deficiencies, their functional integrity, in terms of the parameters studied, is decidedly dissimilar with impaired function in vitamin B<sub>12</sub> deficiency and essentially normal function in folic acid deficiency.

#### MATERIALS AND METHODS

Segmented neutrophils (PMN leukocytes) from normal controls and from vitamin-deficient patients were prepared<sup>9</sup> and quantitated. Differential counts of the WBC preparation were done from Wright's stained smears and showed 85%-90% PMN leukocytes. Complete blood counts including red cell indices and bone marrow aspirates from the posterior superior iliac crest were examined in all of the vitamin-deficient patients. Each of the three patients with folic acid deficiency had a marked macrocytic anemia at the time of initial diagnosis. All were thrombocytopenic, and all had a frankly megaloblastic bone marrow and showed polymorphonuclear hypersegmentation. Folic acid levels were below the lower limits of normal for the laboratory in two of the three patients (<2.5 ng/ml). The original specimen of the third patient was lost. All three had normal vitamin B<sub>12</sub> levels. These patients responded to folic acid therapy with a characteristic reticulocytosis with improvement of the anemia. The patients with vitamin B<sub>12</sub> deficiency were diagnosed on the basis of low serum vitamin B<sub>12</sub> values and abnormal Schilling tests. One of the four (R.R.) was not anemic at the time of his diagnosis, but he had a megaloblastic bone marrow, hypersegmented polymorphonuclear leukocytes, and vitamin B<sub>12</sub> value of 57 pg/ml. Two of these patients, E.H. and R.R., were vitamin B<sub>12</sub> deficient on the basis of prior bowel surgery resulting in lack of vitamin B<sub>12</sub> absorption. One of the patients, D.C., was a juvenile diabetic who had a markedly deficient diet while C.J. had characteristic pernicious anemia.

Bacteria for assessment of phagocytosis and microbicidal activity were prepared from an 18-hr culture of *S. aureus* grown in trypticase soy broth, washed and diluted to an appropriate concentration. Bacteria for use in metabolic studies were heat killed at 60°C for 30 min. Phagocytic activity was evaluated from Wright's stained smears prepared 30 and/or 120 min after introducing either two or ten staphylococci per cell.

Hexose monophosphate shunt activity at rest and during uptake of staphylococci or latex particles was determined from the evolution of <sup>14</sup>CO<sub>2</sub> after 30 min of incubation in the presence of 1-<sup>14</sup>C glucose as previously described.<sup>9</sup> Microbicidal activity was determined from colony counts of a suitably diluted 0.01-ml aliquot taken at time 0 and at 30, 60, and 120 min after the addition of bacteria. The cell ratio of these leukocyte-bacteria suspensions was 1:2.

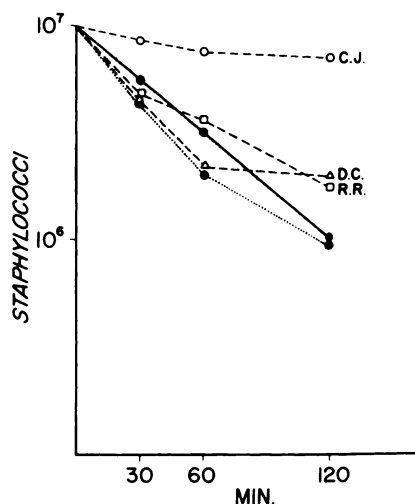


Fig. 1. The mean microbicidal activity of 20 normal controls at 30, 60, and 120 min is shown by the solid line. The shaded area indicates 2 SD above and below the mean. The microbicidal activity of leukocytes from three of the four patients with vitamin B<sub>12</sub> deficiency are shown by dashed lines. After 2 hr of incubation, all had killed fewer staphylococci than normally expected. The mean microbicidal activity of leukocytes from three patients with folic acid deficiency is shown by the dotted line. All were within normal limits.

Significance of differences between normal control and the results from patients with vitamin B<sub>12</sub> and folic acid deficiencies was determined with the Student's *t* test.

## RESULTS

The effects of vitamin B<sub>12</sub> and folic acid deficiencies on the phagocytosis-associated activation of the HMPS and on bacterial killing are shown in Table 1 and Fig. 1. Resting metabolism tended to be low, but it was not significantly lower than normal. With phagocytosis either of latex or of staphylococci, however, there was a statistically significant impairment of HMPS activation to 36% and 35% of control in the leukocytes of patients with vitamin B<sub>12</sub> deficiency, regardless of the cause of the deficiency. Microbicidal activity at 2 hr after inoculation was reduced by more than 2 SD from the mean control level in all three of the four vitamin B<sub>12</sub>-deficient patients where this was measured. The patient with true pernicious anemia (C.J.) showed the greatest impairment of microbicidal activity. Two of these patients, R.R. and E.H., were restudied after receiving vitamin B<sub>12</sub>. Of these, E.H. demonstrated a marked improvement of phagocytosis-associated HMPS activation by the tenth posttreatment day. Initially, with phagocytosis of latex and staphylococci, her leukocytes' HMPS metabolism was 43% and 54% of the mean control value (Table 1). At the tenth posttreatment day, this metabolic activity was 100% of the control. The other patient, at 25 days posttreatment, also showed improvement of leukocyte function from approximately 40% of control to about 60% of control.

The patients with folic acid deficiency did not show a decrease either of phagocytosis-associated HMPS activation or of microbicidal activity.

## DISCUSSION

The primary role of the PMN leukocyte in host defense against bacterial invaders is well recognized. It has been suggested that deficiencies of any one or more of the B-complex vitamins may impair the metabolic activities associated with phagocytosis.<sup>10</sup> Thiamin, riboflavin, and pyridoxine deficiencies all have been associated with decreased activities of enzymes involved in HMPS activity.

In addition, the leukocytes of children with severe protein-calorie malnutrition have decreased metabolic and microbicidal activities,<sup>5</sup> which can be related to decreased activity of granule-bound NADPH oxidase.<sup>6</sup> Another study has demonstrated quantitatively impaired NBT reduction and decreased pyruvate kinase activity with normal phagocytosis in the leukocytes of patients with refractory anemias of unspecified types.<sup>11</sup>

Leukocyte function, in terms of metabolic and microbicidal activity during the course of vitamin B<sub>12</sub> and folic acid deficiencies, has not been specifically investigated. However, two previous studies on leukocyte metabolism in vitamin B<sub>12</sub> deficiency have shown that 3-<sup>14</sup>C propionate oxidation to CO<sub>2</sub> is impaired<sup>4</sup> and that the activity of methylmalonyl CoA mutase is decreased.<sup>3</sup> Other work using newborn animals fed diets deficient in folic acid has demonstrated an increased susceptibility to fatal infections,<sup>12</sup> and low tissue folate levels are suspected of being a predisposing factor in the bacteriuria of pregnancy.<sup>10</sup> Our studies, however, have shown no impairment of phagocytosis-associated metabolism or microbicidal activity in the leukocytes of patients with folic acid deficiency. Only in the four patients with vitamin B<sub>12</sub> deficiency did a marked decrease in the phagocytosis-associated HMPS activation occur. These patients also have shown a slight to moderate impairment of intraleukocytic microbicidal activity. Although both of these defects of PMN function are correctable with specific therapy, it is not possible to assume that they are entirely caused by vitamin B<sub>12</sub> deficiencies. Two of these patients may have possessed multiple deficiencies due to malabsorption, and one of the patients also is diabetic. Only one of the patients had been vitamin B<sub>12</sub> deficient on the basis of a deficiency of intrinsic factor. It is not unreasonable to suppose that, in view of the impaired DNA and protein synthesis in vitamin B<sub>12</sub> deficiency, one or more of the enzymes involved in HMPS activation are reduced in the cell. Since hexokinase and NADP are rate-limiting for HMPS activity,<sup>13</sup> quantitation of these factors or the substances necessary for their formation would seem to be the most potentially productive avenues for future investigation. Our findings of reduced PMN reactivity are not necessarily incompatible with the infrequency of life-threatening infection in vitamin B<sub>12</sub> deficiency. Metabolic activity is reduced by approximately 40% and microbicidal activity only by 5%-10%. Much more profound reductions probably are necessary before serious compromise of PMN functional integrity occurs. The fact that this defect occurs with vitamin B<sub>12</sub> deficiency, but not with folic acid deficiency, suggests a specific role for vitamin B<sub>12</sub> in the production of intermediates necessary for normal cell metabolism and function.

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